21. An expression vector comprising the nucleic acid of claim 14 operably linked to additional elements for expression of a gene of interest.

22. A host cell comprising the expression vector of claim 21.

23. A host cell comprising the nucleic acid of claim 14.

C1 Cont

24. A method of secreting a heterologous polypeptide of interest in a cell comprising using a translational initiation region variant operably linked to nucleic acid encoding said heterologous polypeptide to express said heterologous polypeptide, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region, wherein said translational initiation region includes a prokaryotic secretion signal sequence selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA.--

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REMARKS

In the Response to Restriction Requirement, applicants elected Group II, drawn to a method of optimizing secretion of a heterologous peptide with variants in the translational initiation region. However, this group of claims has already been issued in the immediate parent to this application, U.S. Pat. No. 6,242,177. Therefore, applicants seek herein to replace such claims with the claim set presented herein, namely, claims 5-24. These claims reflect claims 1-6 and 8-21 of U.S. Pat. No. 5,840,523, to which the present application is closely related, except that independent claims 5 and 14 do not contain the language "wherein the amino acid sequence of said translational initiation region variant is not altered," and independent claims 13 and 24 incorporate the Markush language of claim 13 of the '523 patent into claims 10 and 21 thereof, respectively.

Support for these new claims is generally found in the entirety of the specification, the invention being clearly oriented to secretion, and its enhancement, surprisingly via variants of a translational initiation region exhibiting a strength less than that of the wild-type counterpart, and nucleic acids, vectors, and host cells enabling such process.

Specific support for new claims 5, 6, 13, 14, and 21-24 can be found, for example, on page 3, lines 8-16, page 6, lines 24-38, and page 20, lines 13-17 of the specification, with claims 13 and 24 being also supported on at least page 7, lines 1-3. Claims 7-12 and 15-20 are supported at least on page 7, lines 1-3.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

For the Examiner's convenience, a clean copy of the currently pending claims is attached hereto.

If the Examiner has any questions regarding this response, he is invited to call the undersigned attorney at the number indicated below.

Respectfully submitted,
GENENTECH, INC.

Date: January 27, 2003

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 1-4 have been deleted.

New claims 5-23 have been added as follows:

--5. (New) A method of secreting a heterologous polypeptide of interest in a cell comprising using a translational initiation region variant operably linked to nucleic acid encoding said heterologous polypeptide to express said heterologous polypeptide, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region.--

--6. (New) The method of claim 5 wherein the amount of secreted polypeptide when said nucleic acid is operably linked to said variant is greater than the amount of secreted polypeptide when said nucleic acid is operably linked to the wild-type translational initiation region.--

--7. (New) The method of claim 5 wherein said translational initiation region includes a prokaryotic secretion signal sequence.--

--8. (New) The method of claim 7 wherein said secretion signal sequence is selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA.--

--9. (New) The method of claim 8 wherein said signal sequence is selected from the group consisting of STII, PhoE and LamB.--

--10. (New) The method of claim 9 wherein said signal sequence is STII.--

--11. (New) The method of claim 9 wherein said signal sequence is LamB.--

--12. (New) The method of claim 9 wherein said signal sequence is PhoE.--

--13. (New) A nucleic acid encoding a translational initiation region variant, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region, wherein said translational initiation region includes a prokaryotic secretion signal sequence selected from the group consisting of STII, OmpA, PheE, LamB, MBP and PhoA.--

--14. (New) A nucleic acid encoding a polypeptide operably linked to a translational initiation region variant, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region.--

--15. (New) The nucleic acid of claim 14 wherein said translational initiation region includes a prokaryotic secretion signal sequence.--

--16. (New) The nucleic acid of claim 15 wherein said translational initiation region includes a signal sequence selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA.--

--17. (New) The nucleic acid of claim 16 wherein said signal sequence is selected from the group consisting of STII, PhoE and LamB.--

--18. (New) The nucleic acid of claim 17 wherein said signal sequence is STII.--

--19. (New) The nucleic acid of claim 17 wherein said signal sequence is LamB.--

--20. (New) The nucleic acid of claim 17 wherein said signal sequence is PhoE.--

--21. (New) An expression vector comprising the nucleic acid of claim 14 operably linked to additional elements for expression of a gene of interest.--

--22. (New) A host cell comprising the expression vector of claim 21.--

--23. (New) A host cell comprising the nucleic acid of claim 14.--

--24. (New) A method of secreting a heterologous polypeptide of interest in a cell comprising using a translational initiation region variant operably linked to nucleic acid encoding said heterologous polypeptide to express said heterologous polypeptide, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region, wherein said translational initiation region includes a prokaryotic secretion signal sequence selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA.--

CLEAN SET OF PENDING CLAIMS

5. A method of secreting a heterologous polypeptide of interest in a cell comprising using a translational initiation region variant operably linked to nucleic acid encoding said heterologous polypeptide to express said heterologous polypeptide, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region.

6. The method of claim 5 wherein the amount of secreted polypeptide when said nucleic acid is operably linked to said variant is greater than the amount of secreted polypeptide when said nucleic acid is operably linked to the wild-type translational initiation region.

7. The method of claim 5 wherein said translational initiation region includes a prokaryotic secretion signal sequence.

8. The method of claim 7 wherein said secretion signal sequence is selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA.

9. The method of claim 8 wherein said signal sequence is selected from the group consisting of STII, PhoE and LamB.

10. The method of claim 9 wherein said signal sequence is STII.

11. The method of claim 9 wherein said signal sequence is LamB.

12. The method of claim 9 wherein said signal sequence is PhoE.

13. A nucleic acid encoding a translational initiation region variant, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region, wherein said translational initiation region includes a prokaryotic secretion signal sequence selected from the group consisting of STII, OmpA, PheE, LamB, MBP and PhoA.

14. A nucleic acid encoding a polypeptide operably linked to a translational initiation region variant, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region.

15. The nucleic acid of claim 14 wherein said translational initiation region includes a prokaryotic secretion signal sequence.

16. The nucleic acid of claim 15 wherein said translational initiation region includes a signal sequence selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA.

17. The nucleic acid of claim 16 wherein said signal sequence is selected from the group consisting of STII, PhoE and LamB.

18. The nucleic acid of claim 17 wherein said signal sequence is STII.

19. The nucleic acid of claim 17 wherein said signal sequence is LamB.

20. The nucleic acid of claim 17 wherein said signal sequence is PhoE.

21. An expression vector comprising the nucleic acid of claim 14 operably linked to additional elements for expression of a gene of interest.

22. A host cell comprising the expression vector of claim 21.

23. A host cell comprising the nucleic acid of claim 14.

24. A method of secreting a heterologous polypeptide of interest in a cell comprising using a translational initiation region variant operably linked to nucleic acid encoding said heterologous polypeptide to express said heterologous polypeptide, wherein the translational strength of said variant translational initiation region is less than the translational strength of the wild-type translational initiation region, wherein said translational initiation region includes a prokaryotic secretion signal sequence selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA.